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First sexual morph record of *Sarcopodium vanillae*

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Abstract—*Sarcopodium vanillae* was isolated from a dead leaf of *Dracaena* in Chiang Rai Province, Thailand. Combined analyses of ACT, ITS, LSU, and tub2 sequence data obtained from the cultures derived from single spore isolates confirm that our collections represent *S. vanillae*. This is the first record of the sexual morph, and the first record of *S. vanillae* from *Dracaena*. A description and illustrations of both sexual and asexual stages of *S. vanillae* are provided.

Key words—multigene, new host record, *Nectriaceae*, saprobe, taxonomy

Introduction

During our research on the diversity of microfungi in Thailand, we recovered isolates from *Dracaena* in Chiang Rai Province that were typical of *Nectriaceae* based on our preliminary morphological studies. Further morphological and molecular characterization (multigene-based phylogeny of nuclear ribosomal and protein-coding loci ACT, ITS, LSU and Tub2 sequence data) revealed the taxon as *Sarcopodium vanillae*. *Nectriaceae* (*Hypocreales;* Rossman & al. 1999, Maharachchikumbura & al. 2015) is typified by a characteristic perithecial wall structure and specific anamorphic

states. Its uniloculate perithecioid ascomata are yellow, orange, red, purple or white, and the asexual morph is phialidic (Rogerson 1970, Rehner & Samuels 1995). The genus *Sarcopodium* Ehrenb. has had numerous nomenclatural changes since it was first described in 1818 that have been synonymised under *Sarcopodium*.

Cyphina Sacc., had already been synonymised under *Sarcopodium* by Sutton (1977) and under "one fungus: one name" by Rossman & al. (2016) supported the placement of the (asexual) *Actinostilbe* and the (sexual) *Lanatonectria* under the much earlier (asexual) *Sarcopodium*. *Sarcopodium* Ehrenb. is closely related to *Pseudonectria* Seaver. (Rossman & al. 1999). Sutton (1981) synonymized *Actinostilbe* Petch. under *Sarcopodium*; and all five species of *Lanatonectria* Samuels & Rossman (a genus described from a sexual morph) have been transferred to *Sarcopodium* following phylogenetic analyses (Lombard & al. 2015, Pennycook & Kirk 2019a). The new epithet for the type species ("*flocculentum*") has priority over the previous heterotypic synonym, *Sarcopodium macalpinei* (Pennycook & Kirk 2019b).

Some *Sarcopodium* species have both sexual and asexual morphs, but *S. vanillae* has been known only from its asexual morph (Sutton 1981; Rossman 1983, 1996, 2000; Rossman & al. 1993, 2013, 2016). This paper describes the first record of a sexual morph for *S. vanillae* and reports *Dracaena* as a new host record for this fungus.

Materials & methods

Sample collection, morphological study and isolation

Dead leaves of *Dracaena* were collected from Chiang Rai Province in Thailand during November 2017. The samples were taken to the laboratory in Zip-lock bags. Micro-morphological structures were observed and photographed with a Canon EOS 600D digital camera fitted to a Nikon Eclipse Ni compound microscope. Single spore isolates were obtained using the method described in Chomnunti & al. (2014). Germinated spores were transferred to potato dextrose agar (PDA) plates and incubated at 25–30 °C. Growth rates and culture characteristics were recorded after three weeks. The morphological characters were measured using Tarosoft (R) Image Frame Work v. 0.9.7 software. Figures were processed with an Adobe Photoshop CS6 Extended v. 10.0 software. The cultures are deposited in Mae Fah Luang University Culture Collection, Chang Rai, Thailand (MFLUCC). Specimens are deposited in the herbarium of Mae Fah Luang University, Chang Rai, Thailand (MFLU).

DNA extraction, PCR amplification and sequencing

Fungal isolates were grown on PDA for 3–4 weeks at 30 °C. The genomic DNA was extracted from mycelium using the E.Z.N.A. Forensic DNA Kit. Amplification

Table 1. Sequences from *Pseudonectria* and *Sarcopodium* species (and *Stachybotrys chartarum* outgroup) used in the phylogenetic analysis

Taxon	Culture no.	GenBank accession no.			
		ACT	ITS	LSU	tub2
P. buxi	CBS 324.53	KM231171	KM231778	KM231644	KM232037
P. foliicola	CBS 122566	KM231170	KM231777	KM231643	KM232036
S. circinatum	CBS 587.92	KM231180	KM231787	KM231651	KM232046
	CBS 100998	KM231179	KM231786	KM231650	KM232045
S. circinosetiferum	CBS 100251	KM231175	KM231782	KM231646	KM232041
S. flavolanatum	CBS 112283	KM231178	KM231785	KM231649	KM232044
	CBS 128370	KM231177	KM231784	KM231648	KM232043
S. macalpinei	CBS 115296	KM231176	KM231783	KM231647	KM232042
S. vanillae	CBS 100582	KM231173	KM231780	HQ232174	KM232039
	MFLU 17-2595	**MK692541**	**MK608516**	**MK691503**	**MK962543**
	MFLU 17-2597	**MK692542**	**MK685870**	**MK691502**	**MK692544**
Stachybotrys chartarum	CBS 129.13	KM231268	KM231858	KM231738	KM232127

New sequences are set in **bold** font.

primers used were: internal transcribed spacer (ITS)—ITS5 and ITS4 (White & al. 1990); 28S large subunit ribosomal RNA (LSU)—LROR and LR5 (Rehner & Samuels 1994, Vilgalys & Hester 1990); partial actin gene (ACT)—ACT-512F and ACT-783R (Carbone & Kohn 1999); partial beta-tubulin gene (tub2)—Bt-2a and Bt-2b (Glass & Donaldson 1995). The PCR mixer comprised 1 µl forward primer, 1 µl reverse primer, 9.5 µl distilled deionized (DD) water, and 12.5 µl mixer. The PCR conditions for ITS and LSU were 3 min at 94 °C; followed by 35 cycles of 30 s at 94 °C, 50 s at 55 °C, and 90 s at 72 °C; and a final elongation step at 72 °C for 10 min. Conditions for ACT were an initial elongation step of 2 min at 95 °C; followed by 35 cycles of 45 s at 95 °C, 45 s at 55 °C, and 1 min at 72 °C; and a final elongation step of 10 min at 72 °C. Conditions for tub2 were an initial 8 min of 95 °C; followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C, and 1 min at 72 °C; and a final elongation step at 72 °C for 5 min. The PCR products were purified and sequenced at Shanghai Sangon Biological Engineering Technology and Service Co. Isolates including accession numbers of gene sequences are listed in Table 1.

Phylogenetic analysis

Sequence data of *Sarcopodium vanillae* and related taxa (Table 1) were downloaded from GenBank following Zeng & al. (2018) and Yang & al. (2018). The multiple sequence alignments were produced with MAFFT v. 7 (http://mafft.cbrc.jp/

alignment/server/index.html) and BioEdit v. 7.0.5.2 (Hall 1999). The phylogenetic analyses were performed using maximum likelihood (ML) trees and generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis & al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller & al. 2010) using GTR+I+G model of evolution. Parsimony analysis was carried out with the heuristic search option in PAUP v. 4.0b10 (Swofford 2002) using the following parameters: characters unordered with equal weight, random taxon addition, branch swapping with tree bisection-reconnection (TBR) algorithm, branches collapsing if the maximum branch length was zero. Alignment gaps were treated as missing characters in the combined data set, where they occurred in relatively conserved regions. Trees were inferred using the heuristic search option with 1000 random sequence additions, with maxtrees set at 1000. Descriptive tree statistics for parsimony; tree length (TL), consistency index (CI), retention index (RI), relative consistency index (RC) and homoplasy index (HI) were calculated for trees generated. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed to determine whether trees were significantly different. Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) to evaluate posterior probability (PP) (Rannala & Yang 1996) by Markov chain Monte Carlo sampling. GTR+I+G was used in the command. Six simultaneous Markov chains were run for 2,000,000 generations with trees sampled every 200th generation. The distribution of log-likelihood scores was examined to determine stationary phase for each search and to decide if extra runs were required to achieve convergence, using the program Tracer 1.4 (Rambaut & Drummond 2007). First 10% of generated trees were discarded and remaining 90% of trees were used to calculate posterior probabilities of the majority rule consensus tree. The phylogenetic tree was figured in FigTree v. 1.4 (Rambaut 2014) and edited using Microsoft Office Power Point 2007. Sequences derived in this study were deposited in GenBank (Table 1).

Phylogenetic results

The combined sequence alignments comprised 12 isolates, with *Stachybotrys chartarum* (CBS 129.13) as the outgroup taxon. The combined dataset comprised 3065 characters including alignment gaps, of which 844 were derived from ITS, 624 from LSU, 812 from ACT, and 785 from tub2. The MP analysis for the combined dataset had 415 parsimony informative, 2400 constant, 250 parsimony uninformative characters and yielded a single most parsimonious tree (TL = 1109, CI = 0.812, RI = 0.761, HI = 0.188, RC=0.617; Fig 1). The RAxML analysis of the combined dataset yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value of –9266.358949. The matrix had 656 distinct alignment patterns, with 17.28% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.220970, C = 0.279441, G = 0.267083, T = 0.232506; substitution rates

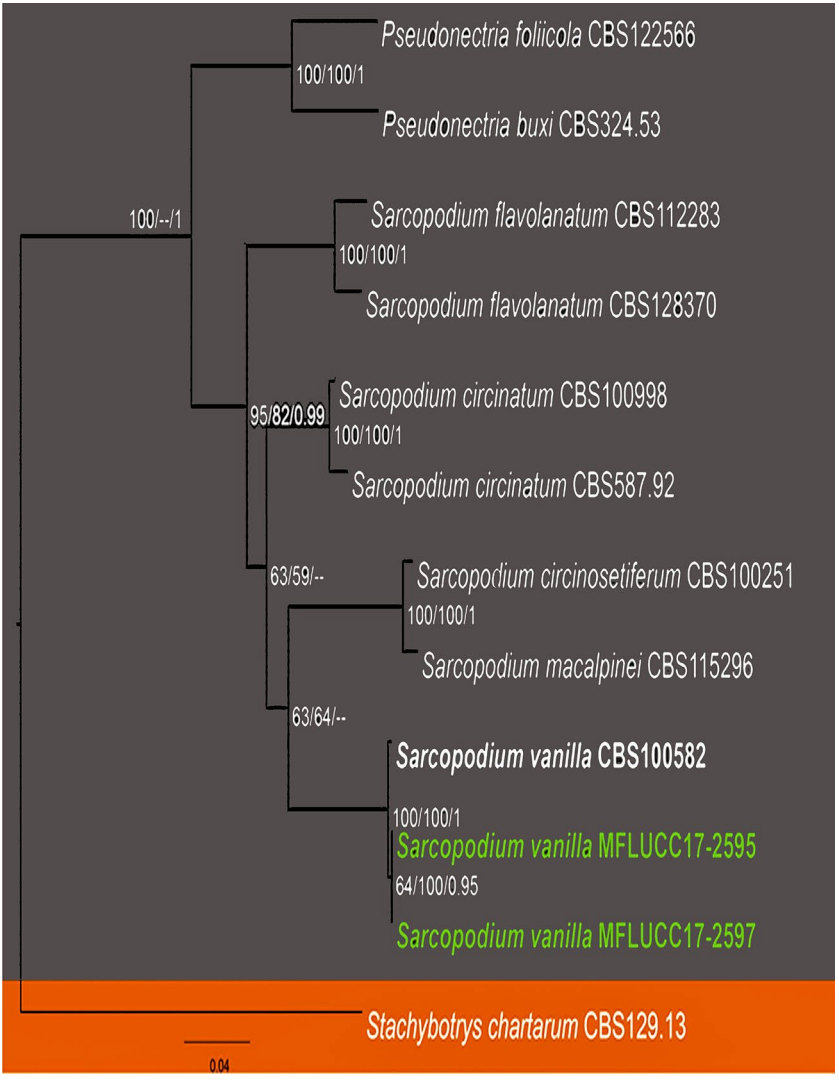

Fig. 1. Phylogram generated from RAxML based on combined ITS, LSU, ACT, and Tub2 sequence data. Bootstrap support values for maximum likelihood (ML) ≥60%, maximum parsimony (MP) ≥60%, and Bayesian posterior probabilities (PP) ≥0.95 are indicated as ML/MP/PP at the nodes. Ex-type strains are in bold and the strains from this study are indicated in green.

AC = 0.730175, AG = 1.879094, AT = 1.313827, CG = 0.798328, CT = 4.086170, GT = 1.000000; gamma distribution shape parameter α = 0.147281.

Bayesian posterior probabilities from Bayesian inference analysis were assessed with a final average standard deviation of split frequencies = 0.0056. The phylogenetic tree in this study showed that our strains (*Sarcopodium vanillae* MFLUCC 17-2595 and MFLUCC 17-2597) grouped in the *Sarcopodium* clade, and formed a well-supported cluster with *S. vanillae* (CBS 100582) with 64% ML, 100% MP, and 0.95 PP.

Taxonomy

Sarcopodium vanillae (Petch) B. Sutton,
Trans. Brit. Mycol. Soc. 76: 99 (1981) Plates 1, 2
≡ *Actinostilbe vanillae* Petch, Ann. Roy. Bot. Gard. (Peradeniya) 9(3):327 (1925)

Saprobic on dead leaves of *Dracaena* (*Asparagaceae*).

Sexual morph: Ascomata 150–200 μm high, 160–240 μm wide (x = 173 × 189 μm, n = 5), perithecial, subglobose, solitary or in groups, soft-textured, pale yellow or rarely orange, superficial on a leaf or erumpent, with a papillate ostiole. Peridium 15–25 μm wide (x = 47 μm, n = 5), composed of several layers of white to light orange cells of textura angularis. Asci 36–52 × 3–5 μm (x = 44 × 4.5 μm, n = 20), 4-spored, unitunicate, cylindrical, rounded at apex, clavate to fusiform, short pedicellate. Ascospores 8–12 × 3–4.5 μm (x = 11 × 3.9 μm, n = 20), fasciculate, broadly elongate, 1-septate.

Asexual morph: Mycelium, branched, septate, hyaline, smooth. Conidiomata 200–210 × 220–240 μm (x = 205 × 230 μm, n = 5), sporodochial, solitary or gregarious, setiferous, yellow to bright yellow or rarely orangish brown, soft-textured, superficial, separate, gregarious or confluent, sessile, attached to the substratum by a small stroma concentrated in the epidermis or outer layers of peridermal tissue, pulvinate, setose. Setae 100–110 × 5–10 μm (x = 105 × 7.5 μm, n = 5), septate, unbranched, cylindrical, incurved, erect, very thick-walled, medium to pale golden brown, more or less straight (occasionally slightly bent either at the base or nearer the apex), erect, hyaline, pointed or rounded at the apex. Conidiophores mononematous, verticillately or penicillately branched, straight or flexuous, smooth, hyaline, short, septate, with 1–4 monochasial branching, compactly arranged, cylindrical, intermixed with long setae. Conidiogenous cells enteroblastic, monophialidic, integrated, cylindrical or more frequently tapered towards the apices, subulate, widest from middle to base, 10–20 × 1.2–2.7 μm (x = 15 × 1.95 μm, n = 5), with inconspicuous collarette, hyaline, smooth,

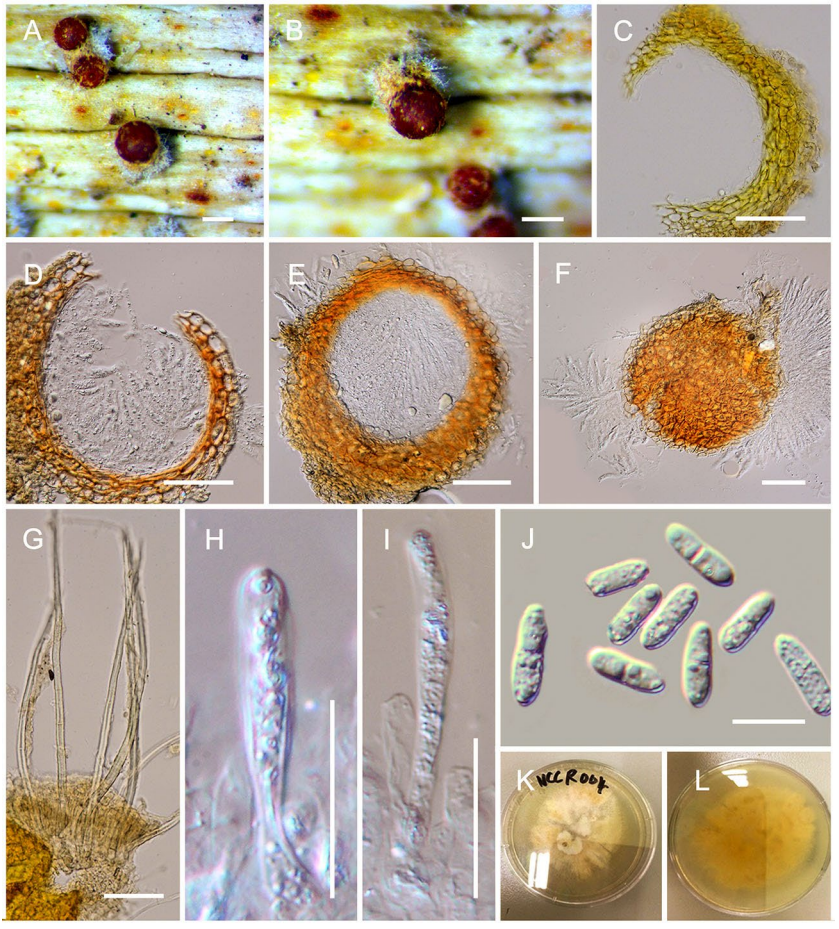

Plate 1. *Sarcopodium vanillae* (MFLU 19-0567, herbarium specimen): A, B. Perithecia on host surface; C–E. Perithecial cross sections; F. Crush mount of perithecium; G. Conidioma on perithecium; H, I. Asci, J. Ascospores; K, L. PDA cultures. Scale bars: A, B = 200 μm; C–G = 50 μm; H, I = 20 μm; J = 10 μm

formed as the ultimate branches of conidiophores and completely covering the external face of the conidiomata. Conidia 5–9 × 2.1–2.6 μm (x = 6 × 2.5 μm, n = 20), cylindrical, 0–1-septate, smooth, hyaline, rounded at both ends, held together in a slimy mass, ellipsoid to oval, straight.

Culture Characteristics: Colony on PDA reaching 30–40 mm diam. after 3 weeks at 25–30 °C; from above, white to yellow at margin, white to

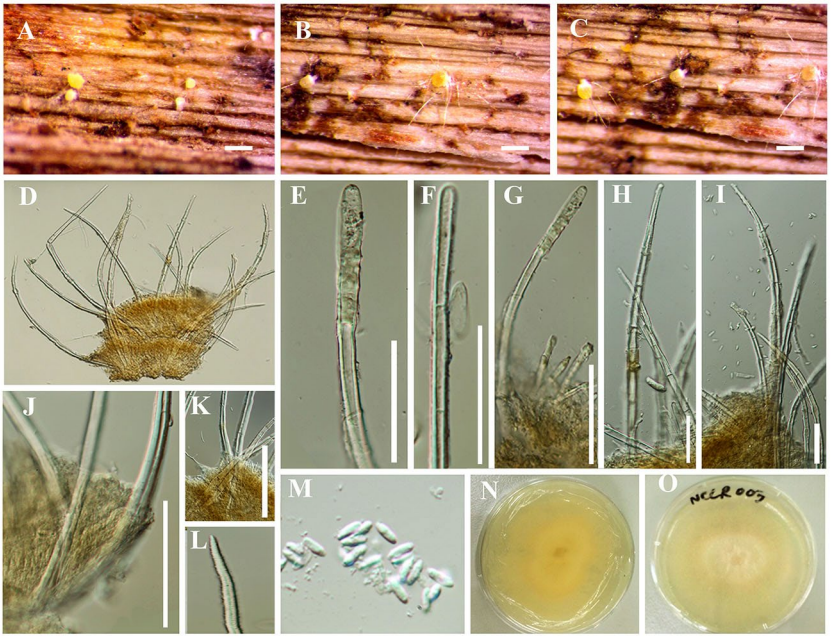

Plate 2. *Sarcopodium vanillae* (MFLU 19-0566, herbarium specimen): A–C. Conidiomata on host surface; D. Conidioma; E–I. Setae; J, K. Setal bases; L. Setal apex; M. Conidia; N, O. PDA cultures. Scale bars: A–C = 200 µm; D, J, K = 100 µm; E–I, L, M = 50 µm.

orange in the middle, white at centre; from below, yellow, medium dense, irregular, slightly raised to umbonate, surface slightly rough, dull with umbonate edge, concave at centre, fluffy to floccose, with white tufts at centre.

Specimens examined: **THAILAND**, **Chiang Rai Province**, **Mae Lao District**, on dead leaf of *Dracaena*, 17 November 2017, Napalai Chaiwan NCCR003 (MFLU 19-0566 [asexual morph]; living culture MFLUCC 17-2595); Napalai Chaiwan NCCR004 (MFLU 19-0567 [sexual + asexual morph]; living culture MFLUCC 17-2597).

Discussion

Our strains share similar morphological characters with *S. vanillae* strain CBS 100852, which was isolated from *Anthurium* sp. in Ecuador (Lombard & al. 2015). The phylogenetic analysis also supports the close relationship. The genus *Sarcopodium* has both sexual and asexual morphs (Wijayawardene & al. 2017a,b, 2018). The conidial morphology of our strain is similar to *S. circinatum* (the type species of the genus); however our conidiomata and conidiophores more closely resemble *Volutella ciliata* (CBS 483.61)

(Lombard & al. 2015). Previously, only the asexual morph has been observed for *S. vanillae* (Sutton 1981), and this study is the first report of the sexual morph. *Sarcopodium vanillae* has been reported from *Abelmoschus manihot* in Papua New Guinea, *Citrus nobilis* in Brunei, *Vanilla planifolia* in Sri Lanka, and *V. tahitensis* in Papua New Guinea (Farr & Rossman 2019). This study provides the first report of *S. vanillae* from *Dracaena*, and its first report from Thailand.

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